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Tuber in China: *T. microspermum* and *T. microspiculatum* spp. nov.

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Abstract — Two new species, *Tuber microspermum* and *T. microspiculatum,* are described from China. *Tuber microspermum* is characterized by small asci and spiny reticulate ascospores; *T. microspiculatum* can be separated from the other species with spinoreticulate ornamented ascospores by numerous minute meshes.

Key words — *Ascomycota*, *Tuberaceae*, truffle

Introduction

China is especially rich in species of *Tuber*. Since the first publication on this genus describing a new species from China, *T. taiyuanense* B. Liu (Liu 1985), many previously described and new species have been published (Wang 1988; Wang & Li 1991; Wang et al. 1998; Wang & He 2002; Tao et al. 1989; Chen et al. 2005; Chen & Liu 2007; Fan et al. 2011). Because all *Tuber* species grow underground, it can be very difficult to find specimens. Therefore, the number of species in China is inevitably underestimated. After continuing investigation of the genus in China, we describe and illustrate two additional new species.

Materials & methods

The materials were collected from the local mushroom market in Kunming, China. The specimens are deposited in BJTC (Herbarium Biology Department, Capital Normal University). Macroscopic characters were described from fresh specimens. Microscopic characters were described from razor-blade sections of fresh specimens mounted in 3% KOH; sections were stained with Melzer's reagent, rinsed, and mounted in polyvinyl lactic glycerol to make permanent slides for archiving with dried specimens. For scanning electron microscopy (SEM), ascospores were scraped from the dried gleba onto doubled-sided tape, which was mounted directly on an SEM stub, coated with gold-palladium, and examined and photographed with a HITACHI S-4800 SEM.

Taxonomy

Tuber microspermum L. Fan & J.Z. Cao, **sp. nov.** Figs. 1–4

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A yellow brown truffle with small asci and small spinoreticulate ascospores that differs from *Tuber pseudoexcavatum* in an ascoma that lacks an excavated base.

Type: China. Yunnan Province, Kunming, from the local mushroom market. 20 Dec. 2010, Jin-zhong Cao 112 (**Holotype**, BJTC FAN149).

Etymology: *microspermum* (Lat.), referring to the small ascospores.

Ascomata 1.5 cm diam., subglobose or slightly lobed, firm, solid, surface smooth to partially minutely verrucose, glabrous, yellow brown at maturity. Odor slight. Peridium 200–300 µm thick, two layers; outer layer 80–120 µm thick, pseudoparenchymatous, composed of small subangular or subglobose cells mostly 7.5–12.5 µm in diam., with thickened and brown walls, darker towards the outer surface; inner layer composed of intricately interwoven hyphae, hyaline, thin-walled, branched, septate, 2.5–5 µm in diam. Gleba brown to dark brown at maturity, marbled with numerous, narrow, branched, white to white-yellow veins. Asci subglobose, ellipsoid or irregular, hyaline, thick-walled (3–5 µm), 45–70 × 40–60 µm, mostly with a short stalk, 1–5 spores. Ascospores ellipsoid to broad ellipsoid, dark brown at maturity, 15–25(–27.5) × 12.5–20 µm in 2–5-spored asci (30–35 × 22.5–25 µm in 1-spored asci) excluding ornamentation; ornamentation distinctly spinoreticulate, spines 4–6 µm high, straight mostly, the meshes generally 3–6 across the spore width.

Comments — *Tuber microspermum* is characterized by the small ascospores and asci. In ascospore pattern the new species resembles *T. pseudoexcavatum,* which is easily distinguished by a different ascoma that is typically excavated at the base (Wang et al. 1998). Other closely related *Tuber* species with spinyreticulate spores are *T. taiyuanense* and *T. huidongense* (Liu 1985; Wang & He 2002), which can be separated from *T. microspermum* by their larger ascospores and asci.

Tuber microspiculatum L. Fan & Yu Li, **sp. nov.** Figs 5–8

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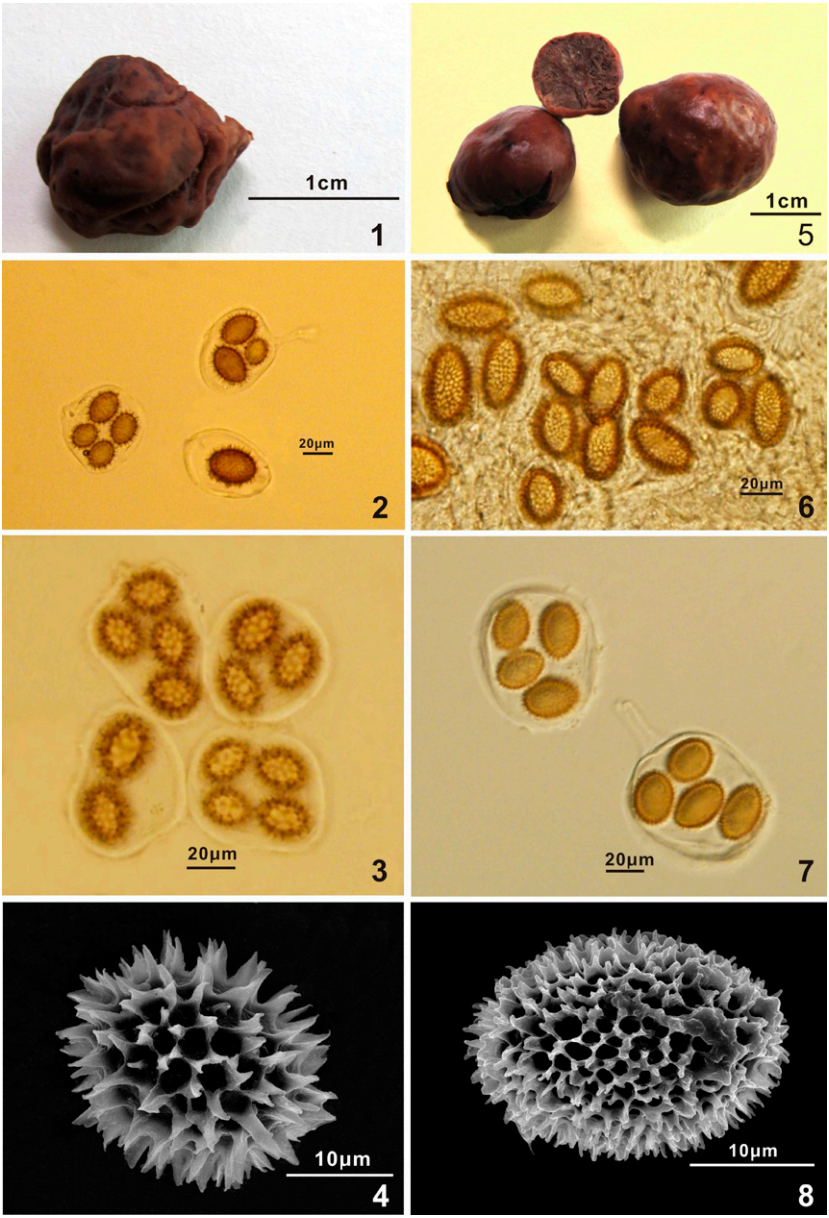
Differs from all other *Tuber* species with spinoreticulate ascospores by an ornamentation of numerous minute narrow spine-like meshes.

Type: China. Kunming, Yunnan Province, from the local mushroom market. 20 Dec. 2010, Jin-zhong Cao 101 (**Holotype,** BJTC FAN138).

Etymology: *microspiculatum* (Lat.), referring to the numerous minute meshes of the spinoreticulate spore ornamentation.

Ascomata 2–2.5 cm in diam., globose or subglobose, firm, solid, with or without distinct umbilicate depression at the base, surface smooth and



Figs 1–8. ***Tuber microspermum*** (BJTC FAN149, holotype): 1. Ascocarp; 2–3. Asci and ascospores observed under light microscope; 4. Ascospore observed under SEM. ***Tuber microspiculatum*** (BJTC FAN138, holotype): 5. Ascocarps; 6–7. Asci and ascospores observed under light microscope; 8. Ascospore observed under SEM.

glabrous, white yellow, pale yellow or light brown, reddish brown at maturity. Odor slight, not pungent. Peridium 200–250 μm thick, two layers; outer layer 50–100 μm, pseudoparenchymatous, composed of small subangular or subglobose cells, mostly 7.5–15 μm in diam., with slightly thickened and yellowish-brown walls, darker towards the outer surface; inner layer composed of intricately interwoven hyphae, hyaline, thin-walled, branched, septate, 2.5–5 μm in diam. Gleba brown to nearly reddish brown at maturity, marbled with numerous, narrow, branched, white-yellow veins radiating from the base. Asci subglobose, ellipsoid or irregular, 60–85 × 55–70 μm, sessile or with a short stalk, (1–)2–4 spores. Ascospores ellipsoid, a few broad ellipsoid to subglobose, light brown to yellow-brown at maturity, (20–)22.5–35(–40) × (15–)17.5–22.5 μm in 2–4-spored asci and 42.5–45 × 22.5–25 μm in 1-spored asci excluding ornamentation; ornamentation of spines 2.5–4(–5) μm high, mostly connected at bases by an alveolate reticulum, the meshes minute and numerous, generally 10–16 across the spore width, a few of large spores in 1-spored asci sometimes with 4–8 meshes across the spore width.

Comments — *Tuber microspiculatum* differs from all related species with spiny-reticulate ascospores by spore ornamentation with very minute and numerous meshes, which are so narrow that they can be easily confused with the spines under light microscope. SEM observation (Fig. 8) is helpful in clarifying the characteristics.

Species of the *T. rufum* complex, which appear similar to *T. microspiculatum* under the light microscope, can be separated by the presence of isolated spines on the ascospore surface.

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